ALKYNES I

Field of the invention

The present invention is directed to novel compounds, to a process for their preparation, their use in therapy and pharmaceutical compositions comprising said novel compounds.

Background of the invention

The metabotropic glutamate receptors (mGluR) are G-protein coupled receptors that are involved in the regulation and activity of many synapses in the central nervous system (CNS). Eight metabotropic glutamate receptor subtypes have been identified and are subdivided into three groups based on sequence similarity. Group I consists of mGluR1 and mGluR5. These receptors activate phospholipase C and increase neuronal excitability. Group II, consisting of mGluR2 and mGluR3 as well as group III, consisting of mGluR4, mGluR6, mGluR7 and mGluR8 are capable of inhibiting adenylyl cyclase activity and reduce synaptic transmission. Several of the receptors also exist in various isoforms, occurring by alternative splicing (*Chen, C-Y et al., Journal of Physiology (2002), 538.3, pp. 773-786; Pin, J-P et al., European Journal of Pharmacology (1999), 375, pp. 277-294; Bräuner-Osborne, H et al. Journal of Medicinal Chemistry (2000), 43, pp. 2609-2645; Schoepp, D.D, Jane D.E. Monn J.A. Neuropharmacology (1999), 38, pp. 1431-1476*).

The lower esophageal sphincter (LES) is prone to relaxing intermittently. As a consequence, fluid from the stomach can pass into the esophagus since the mechanical barrier is temporarily lost at such times, an event hereinafter referred to as "reflux".

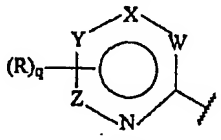
Gastro-esophageal reflux disease (GERD) is the most prevalent upper gastrointestinal tract disease. Current pharmacotherapy aims at reducing gastric acid secretion, or at neutralizing acid in the esophagus. The major mechanism behind reflux has been considered to depend on a hypotonic lower esophageal sphincter. However, e.g. *Holloway & Dent (1990) Gastroenterol. Clin. N. Amer. 19, pp. 517-535,* has shown that most reflux episodes occur

during transient lower esophageal sphincter relaxations (TLESRs), i.e. relaxations not triggered by swallows. It has also been shown that gastric acid secretion usually is normal in patients with GERD.

The problem underlying the present invention was to find new compounds useful in the treatment of GERD.

WO 01/16121 A1 discloses a compound A-L-B, where

A is a 5-, 6- or 7-membered heterocycle

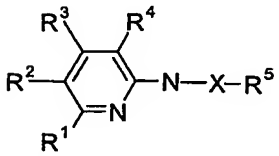


;

L is an alkenylene, alkynylene or azo; and

B is a hydrocarbyl; cyclohydrocarbyl; heterocycle (optionally containing one or more double bonds); or aryl. These compounds have been described as being useful in inter alia cerebral ischemia, chronic neurodegeneration, psychiatric disorders, epilepsy and diseases of the pulmonary system as well as the cardiovascular system.

WO 99/02497 A2 discloses compounds of the formula

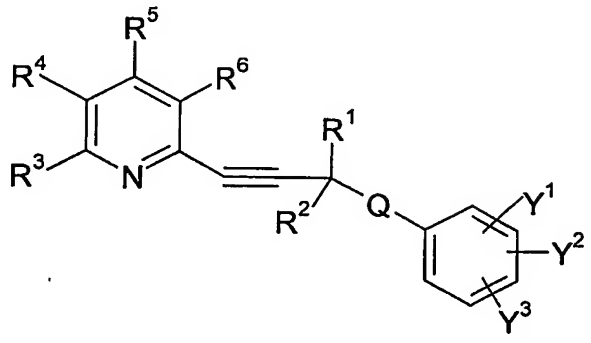


wherein X may be an alkenylene or an alkynylene bonded via vicinal unsaturated carbon atoms, or an azo group; and R^5 may be an aromatic or heteroaromatic group. These compounds have been described as being useful in inter alia epilepsy, cerebral ischemia and Alzheimer's disease.

WO03/022846 A1 discloses *inter alia* the compound 4-(4-pyridin-2-yl-but-3-ynyl)-benzonitrile. The compound is an intermediate in a process for producing compounds useful for treating cancer.

Outline of the invention

The present invention is directed to novel compounds according to the general formula I:



(I)

wherein

R^1 is selected from hydrogen, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, aryl and heteroaryl, wherein the aryl or heteroaryl may be substituted by C_1-C_4 alkyl;

R^2 is selected from hydrogen and C_1-C_4 alkyl;

R^3 is selected from hydrogen, C_1-C_4 alkyl, F, CF_3, CHF_2 and CH_2F;

R^4 is selected from hydrogen, F, CF_3, CHF_2, CH_2F and CH_3;

R^5 is selected from hydrogen and F;

R^6 is selected from hydrogen and F;

Q is selected from C_1-C_4 alkyl, optionally substituted by C_1-C_4 alkyl or C_1-C_4 alkoxy;

Y^1 is selected from hydrogen; halogen; nitrile; C_1-C_4 alkoxy; C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom; benzyloxy; nitro in the meta or para position; and C_1-C_4 alkyl ester;

Y^2 is selected from hydrogen; halogen; nitrile; C_1-C_4 alkoxy; C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom; and C_1-C_4 alkyl ester;

Y^3 is selected from hydrogen; halogen; nitrile; C_1-C_4 alkoxy; C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom; and C_1-C_4 alkyl ester; or

Y^1 and Y^2 may form an aromatic or non-aromatic ring, optionally substituted by halogen, nitrile, C_1-C_4 alkoxy, C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom, benzyloxy or C_1-C_4 alkyl ester;

as well as pharmaceutically acceptable salts, hydrates, isoforms and/or optical isomers thereof, with the exception of 4-(4-pyridin-2-yl-but-3-ynyl)-benzonitrile.

The general terms used in the definition of formula I have the following meanings:

Halogen is chloro, fluoro, bromo or iodo.

C_1-C_4 alkyl is a straight or branched alkyl group, each independently containing 1, 2, 3 or 4 carbon atoms, for example methyl, ethyl, n-propyl, n-butyl or isopropyl. In one embodiment, the alkyl groups may contain one or more heteroatoms selected from O, N and S. Examples of such groups are methyl-ethylether, methyl-ethylamine and methyl-thiomethyl.

Cycloalkyl is a cyclic alkyl, each independently containing 3, 4, 5 or 6 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

C_1-C_4 alkoxy is an alkoxy group containing 1, 2, 3 or 4 carbon atoms, such as methoxy, ethoxy, n-propoxy, n-butoxy or isopropoxy.

The herein used term aryl means aromatic rings with 6-14 carbon atoms including both single rings and polycyclic compounds, such as phenyl, benzyl or naphtyl.

The term heteroaryl as used herein means aromatic rings with 5-14 carbon atoms, including both single rings and polycyclic compounds, such as imidazopyridine, in which

one or several of the ring atoms is either oxygen, nitrogen or sulphur, such as furanyl or thiophenyl.

Within the scope of the invention are also pharmaceutically acceptable salts of the compounds of formula I as well as isomers, hydrates and isoforms thereof.

Pharmaceutically acceptable salts of the compound of formula I are also within the scope of the present invention. Such salts are for example salts formed with mineral acids such as hydrochloric acid; alkali metal salts such as sodium or potassium salts; or alkaline earth metal salts such as calcium or magnesium salts.

The novel compounds according to the present invention are useful in therapy. In one aspect of the invention said compounds are useful for the inhibition of transient lower esophageal sphincter relaxations (TLESRs) and thus for treatment or prevention of gastro-esophageal reflux disorder (GERD). In further embodiments, the compounds according to the present invention are useful for the prevention of reflux, treatment or prevention of regurgitation, treatment or prevention of asthma, treatment or prevention of laryngitis, treatment or prevention of lung disease and for the management of failure to thrive.

A further aspect of the invention is the use of a compound according to formula I for the manufacture of a medicament for the treatment or prevention of functional gastrointestinal disorders, such as functional dyspepsia (FD). Yet another aspect of the invention is the use of a compound according to formula I for the manufacture of a medicament for the treatment or prevention of irritable bowel syndrome (IBS), such as constipation predominant IBS, diarrhea predominant IBS or alternating bowel movement predominant IBS.

A further aspect of the invention is the use of a compound according to formula I, for the manufacture of a medicament for the inhibition of transient lower esophageal sphincter relaxations, for the treatment or prevention of GERD, for the prevention of reflux, for the

treatment or prevention of regurgitation, treatment or prevention of asthma, treatment or prevention of laryngitis, treatment or prevention of lung disease and for the management of failure to thrive.

Still a further aspect of the invention is a method for the treatment of any one of the conditions mentioned above, whereby a pharmaceutically effective amount of a compound according to formula I above, is administered to a subject suffering from said condition(s).

In one aspect of the invention, the compounds of formula I are useful for the treatment and/or prevention of acute and chronic neurological and psychiatric disorders, anxiety and chronic and acute pain disorders. In a further aspect, said compounds are useful for the prevention and/or treatment of pain related to migraine, inflammatory pain, neuropathic pain disorders such as diabetic neuropathies, arthritis and rheumatoid diseases, low back pain, post-operative pain and pain associated with various conditions including cancer, angina, renal or billiary colic, menstruation, migraine and gout.

The term "isomers" is herein defined as compounds of formula I, which differ by the position of their functional groups and/or orientation. By "orientation" is meant stereoisomers, diastereoisomers, regioisomers and enantiomers.

The term "isoforms" as used herein is defined as compounds of formula I which differ by their crystal lattice, such as crystalline compounds and amorphous compounds.

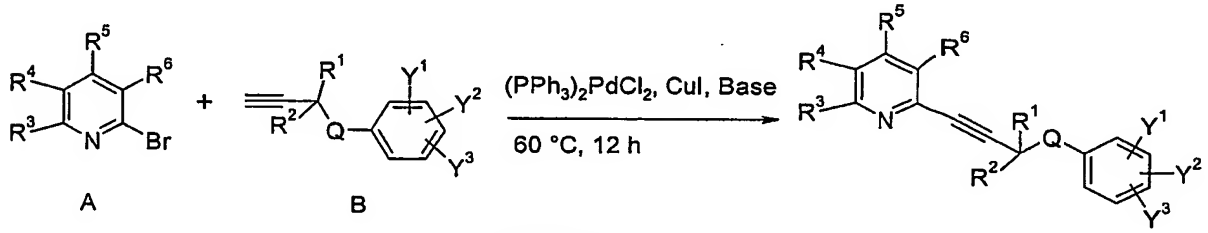
The wording "TLESR", transient lower esophageal sphincter relaxations, is herein defined in accordance with *Mittal, R.K., Holloway, R.H., Penagini, R., Blackshaw, L.A., Dent, J., 1995; Transient lower esophageal sphincter relaxation. Gastroenterology 109, pp. 601-610.*

The wording "reflux" is herein defined as fluid from the stomach being able to pass into the esophagus, since the mechanical barrier is temporarily lost at such times.

The wording "GERD", gastro-esophageal reflux disease, is herein defined in accordance with *van Heerwarden, M.A., Smout A.J.P.M., 2000; Diagnosis of reflux disease. Baillière's Clin. Gastroenterol. 14, pp. 759-774.*

Methods of preparation

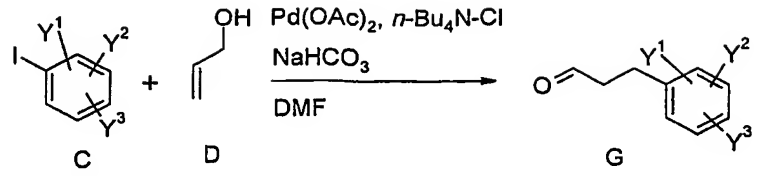
The compounds of formula I above may be synthesized by a Sonogashira coupling (*Tetrahedron Letters* 1975, 50, 4467, S. Thorand, N. Krause *J. Org. Chem.*, **1998**, *63*, 8551-8553, M. Erdélyi, A. Gogoll, *J. Org. Chem.*, **2001**, *66*, 4165-4169) of the aryl bromide A and the alkyne B in the presence of a base such as triethyl amine at room temperature to 60 °C (Scheme 1):



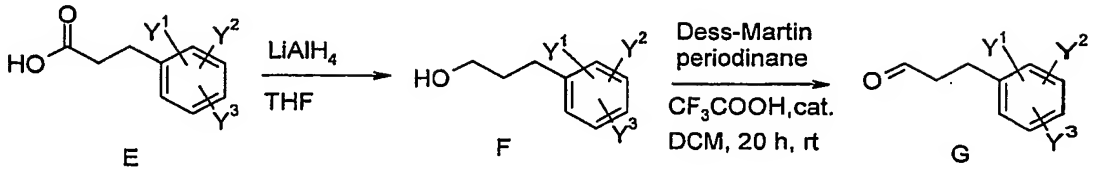
SCHEME 1

In those cases where the terminal alkyne B is not commercially available it is made via the intermediate G, which can be obtained by one of two routes (Scheme 2). One route is by coupling of an aryl iodide C with allyl alcohol D in DMF at room temperature to 60 °C. The other route is by first reducing the carboxylic acid E to the alcohol F using lithium aluminium hydride in THF, starting at 0 °C and ending at reflux temperature and then oxidising the alcohol F to the aldehyde G using Dess-Martin periodinane in DCM at room temperature with a catalytic amount of trifluoroacetic acid.

Route 1:



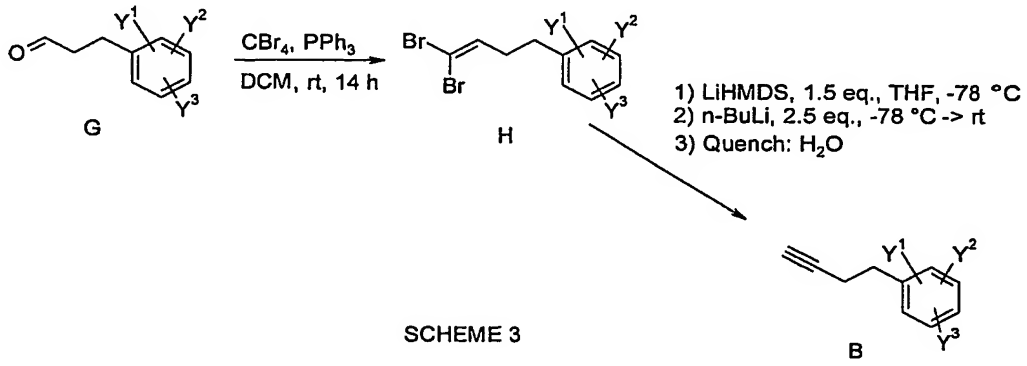
Route 2:



SCHEME 2

With the aldehyde G in hand the alkyne B is made as outlined in scheme 3:

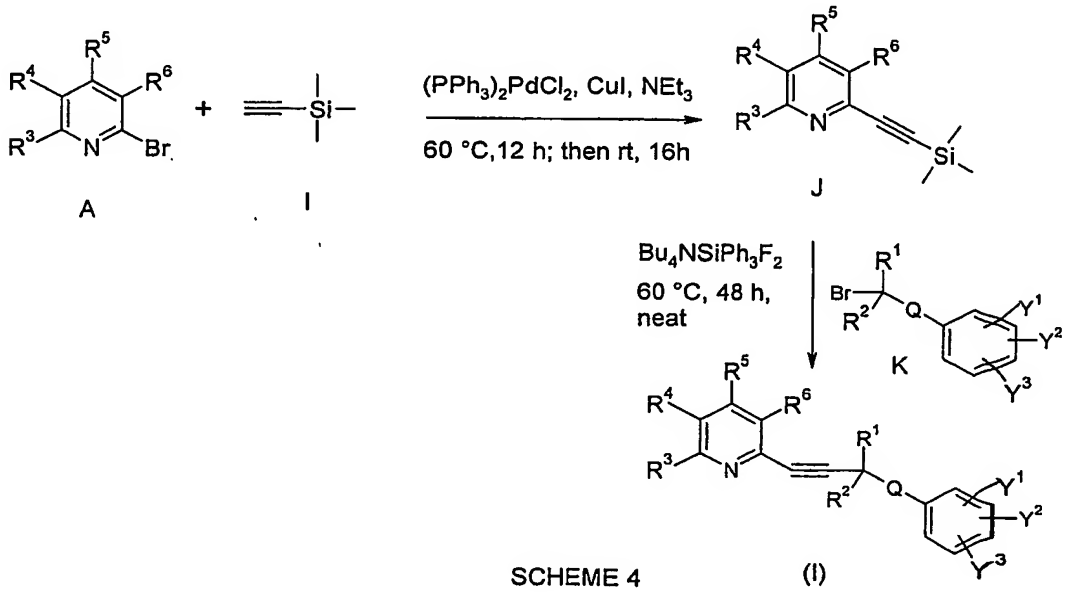
First the aldehyde G is converted to the dibromoalkene H by reaction with tetrabromomethane and triphenylphosphine in DCM at room temperature. Elimination with lithium bis(trimethylsilyl)amide in THF at –78 °C and subsequent halogen-lithium exchange with n-butyl lithium in THF/hexanes at –78 °C to room temperature gives, after quenching with water, the terminal alkyne B. The material B is then used for Sonogashira coupling as outlined in scheme 1.



SCHEME 3

An advantage of this reaction sequence, as shown in schemes 1-3, is that it can be performed without purification of any of the intermediates; purification is only needed after the Sonogashira coupling.

Alternatively, a method that goes via the pyridine J may be used (Scheme 4): Sonogashira coupling of the aryl bromide A with ethynyl(trimethyl)silane I at room temperature to 60 °C in the presence of a base such as triethyl amine gives the pyridine J. The pyridine J is then reacted with the bromide K in the presence of tetrabutylammonium triphenyldifluorosilicate by heating at 60 °C for the appropriate time to obtain compounds of general formula I.



A I J K (I)

SCHEME 4

In the schemes 1, 2, 3 and 4 above, Q, R^1, R^2, R^3, R^4, R^5, R^6, Y^1, Y^2 and Y^3 are defined as for the compounds of formula I above.

Experimental details

DCM is dried over 3Å molecular sieves. THF was distilled from Na/benzophenone just prior to use. All reactions are run under a nitrogen atmosphere. All glassware is dried in at 150 °C for at least two hours prior to its use. Phase separators from International Sorbent Technology (IST) are used. Purification by chromatography is done either on silica gel 60 (0.040-0.063 mm), or by reverse phase chromatography with a C8 column. All NMR spectra are measured in δ-chloroform.

2-bromo-6-methylpyridine is commercially available from Aldrich, $(PPh_3)_2PdCl_2$ from Avacado, Pd $(OAc)_2$ from Aldrich, CuI from Fluka and 4-phenyl-but-1-yn from TCI. If not stated otherwise, the chemicals used are commercially available and are used as such without further purification.

Pharmaceutical formulations

For clinical use, the compounds of formula I are in accordance with the present invention suitably formulated into pharmaceutical formulations for oral administration. Also rectal, parenteral or any other route of administration may be contemplated to the skilled man in the art of formulations. Thus, the compounds of formula I are formulated with at least one pharmaceutically and pharmacologically acceptable carrier or adjuvant. The carrier may be in the form of a solid, semi-solid or liquid diluent.

In the preparation of oral pharmaceutical formulations in accordance with the invention, the compound of formula I to be formulated is mixed with solid, powdered ingredients such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture is then processed into granules or compressed into tablets.

Soft gelatine capsules may be prepared with capsules containing a mixture of the active compound or compounds of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Hard gelatine capsules may contain the active compound in combination with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives or gelatine.

Dosage units for rectal administration may be prepared (i) in the form of suppositories which contain the active substance(s) mixed with a neutral fat base; (ii) in the form of a gelatine rectal capsule which contains the active substance in a mixture with a vegetable oil, paraffin oil, or other suitable vehicle for gelatine rectal capsules; (iii) in the form of a ready-made micro enema; or (iv) in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

Liquid preparations for oral administration may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions, containing the active compound and the remainder of the formulation consisting of sugar or sugar alcohols, and a mixture of ethanol, water, glycerol, propylene glycol and polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, saccharine and carboxymethyl cellulose or other thickening agent. Liquid preparations for oral administration may also be prepared in the form of a dry powder to be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administration may be prepared as a solution of a compound of the invention in a pharmaceutically acceptable solvent. These solutions may also contain stabilizing ingredients and/or buffering ingredients and are dispensed into unit doses in the form of ampoules or vials. Solutions for parenteral administration may also be prepared as a dry preparation to be reconstituted with a suitable solvent extemporaneously before use.

In one aspect of the present invention, the compounds of formula I may be administered once or twice daily, depending on the severity of the patient's condition.

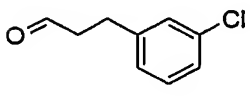
A typical daily dose of the compounds of formula I is from 0.1 – 10 mg per kg body weight of the subject to be treated, but this will depend on various factors such as the route of administration, the age and weight of the patient as well as of severity of the patient's condition.

Examples

Method A

Example 1

Preparation of 3-(3-chlorophenyl)propanal (compound 1):



(1)

Tetrabutylammonium chloride (6.95 g, 0.25 mol, 1.0 eq.) and sodium hydrogen carbonate (5.25 g, 0.625 mmol, 2.5 eq.) were dissolved reasonably in DMF (15 mL) under nitrogen. The mixture was cooled to 0 °C before 3-chloro-iodobenzene (5.96g, 3.10 mL, 0.25 mol), then allyl alcohol (2.18g, 2.56 mL, 0.375 mol, 1.50 eq.) and finally $Pd(OAc)_2$ (0.168g, 7.5 mmol, 0.03 eq.), the latter in small portions, was added. The mixture was stirred at 0 °C for 0.5h, and finally at room temperature for 16h. TLC showed that some 3-chloro-iodobenzene remained. The reaction mixture was then heated at 50°C for 5h. TLC still showed remains of 3-chloro-iodobenzene and consequently, heating at 50°C was continued for another 15h. After that time DMF was evaporated under vacuum with heating at 40°C for 8h.

Afterwards, water (30 mL) was added and the black reaction mixture was extracted with pentane (4x30 mL). Combined pentane phases were dried with sodium sulphate and evaporated. This gave 2.487 g (yield: 59 %) of product.

TLC R_f (heptane/AcOEt 4:1) = 0.17.

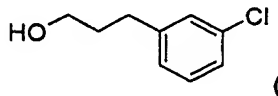
^1H NMR (500 MHz): 9.81 (s, 1H), 7.24-7.17 (m, 3H), 7.09-7.06 (br d, 1H), 2.93 (t, J=7.5 Hz, 2H), 2.78 (t, J=7.5 Hz, 2H).

^{13}C NMR (75 MHz): 200.5, 142.2, 134.0, 129.6, 128.2, 126.3, 126.2, 44.8, 27.6.

Method B

Example 2

Preparation of 3-(3-chlorophenyl)propanol (compound 2):



(2)

3-(3-chlorophenyl)propanoic acid (0.923 g, 5.0 mmol) was dissolved in THF (12 mL) and cooled to 0 °C under nitrogen. Lithium aluminium hydride (0.380 g, 10.0 mmol, 2.0 eq.) was added portionwise. The mixture was allowed to reach room temperature, stirred 0.5h at that temperature and then refluxed for 0.5h.

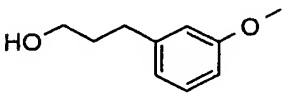
Subsequently, the mixture was cooled and poured onto a saturated solution of tataric acid in ethanol (30 mL) at 0 °C. A 1:1 mixture of sodium sulfate decahydrate and celite (total volume 40 mL) was added. The mixture was stirred for 10 min. and then vacuum filtered through Celite with Et_2O (150 mL). The organic phase was evaporated. This gave a mixture of a clear oil and some white crystals. EtOAc (10 mL) was added. This dissolved the oil, but not the crystals, which were filtered off. The mother liquor was concentrated. In this manner 0.832 g (yield: 98 %) was isolated as a clear oil.

^{1}H NMR (300 MHz): 7.22-7.14 (m, 3H), 7.09-7.03 (m, 1H), 4.29 (s, 1H, br), 3.63 (t, J=6.6 Hz, 2H), 2.66 (t, J=7.4 Hz, 2H), 1.86 (q, J=7 Hz, 2H).

^{13}C NMR (75 MHz): 143.5, 133.6, 129.2, 128.1, 126.2, 125.6, 61.2, 33.5, 31.4.

Example 3

Preparation of 3-(3-methoxyphenyl)propanol (compound 3): prepared according to method B above, using 3-(3-methoxyphenyl)propanoic acid as starting material.

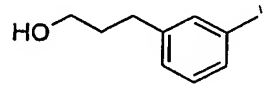


(3)

^{1}H NMR (500 MHz): 7.19 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 7.4 Hz, 1H), 1H, 6.76 (m, 2H), 3.77 (s, 3H), 3.64 (t, J = 6.5 Hz, 2H), 2.66 (t, J = 7.8 Hz, 2H), 2.11 (br s, 1H), 1.87 (br q, **J**= 7.6 Hz, 2H).

Example 4

Preparation of 3-(3-methylphenyl)propanol (compound 4): prepared according to method B above, with 3-(3-methylphenyl)propanoic acid as starting material



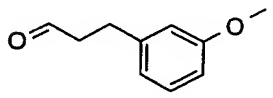
(4)

^{1}H NMR (500 MHz): 7.24 (t, J = 7.5 Hz, 1H), 7.10-7.04 (m, 3H), 3.70 (t, J = 6.6 Hz, 2H), 3.25 (br s, 1H), 2.72 (t, J = 7.6 Hz, 2H), 2.39 (s, 3H), 1.93 (br q, J= 7.6 Hz, 2H).

Method C

Example 5

Preparation of 3-(3-methoxyphenyl)propanal (compound 5):



(5)

Dess-Martin periodinane [1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1H)-one] (1.01 g, 2.38 mmol, 1.1 eq.) was dissolved in DCM (5 mL) at 0 °C. One drop trifluoroacetic acid and then 3-(3-methoxyphenyl)propanol (0.360 g, 2.17 mmol) in DCM (3 mL) were added at 0 °C. When having stirred at room temperature for 20h, the reaction mixture was transferred with Et_2O (25 mL) to 1 M NaOH (10 mL). After stirring for 10 min. the organic phase was separated, then extracted with 1 M NaOH (10 mL) and water

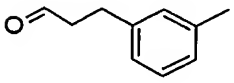
(10 mL), respectively, and dried with sodium sulphate. This gave 0.276 g crude product (77 %), which was used without further purification for the next step.

^{1}H NMR (300 MHz): 9.63 (s, 1H), 7.10-7.02 (m, 1H), 6.70-6.58 (m, 3H), 3.64 (s, 3H), 2.78 (t, J = 7.4 Hz, 2H), 2.60 (t, J = 7.4 Hz, 2H).

^{13}C NMR (300 MHz): 200.9, 159.3, 141.6, 129.2, 120.2, 113.8, 111.1, 54.8, 44.8, 27.9.

Example 6

Preparation of 3-(3-methylphenyl)propanal (compound 6): prepared according to method C above, with 3-(3-methylphenyl)propanol as starting material



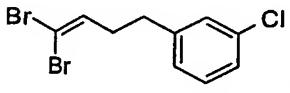
(6)

^{1}H NMR (300 MHz): 9.83 (br t, J = 1.4 Hz, 1H), 7.20 (br t, J = 7.3 Hz, 1H), 7.07-6.98 (m, 3H), 2.94 (t, J = 7.4 Hz, 2H), 2.78 (t, J=7.4 Hz, 2H), 2.34 (s, 3H).

Method D

Example 7

Preparation of 1-chloro-3-(4,4-dibromobut-3-en-1-yl)benzene (compound 7) (Methodology by: E.J. Corey, P.L. Fuchs Tetrahedron Letters **1972**, *No. 36*, 3769-3772.)



(7)

Tetrabromomethane (4.89 g, 14.76 mmol, 2.0 eq.) was dissolved in DCM (45 mL) and then cooled to 0 °C. Triphenylphosphine (3.87 g, 14.76 mmol, 2.0 eq.) was added. The orange solution was stirred 3 min. before the addition of Zn (0.97 g, 14.76 mmol, 2.0 eq.) in small portions. After stirring for 10 min. further, 3-(3-chlorophenyl)propanal (1.24 g,

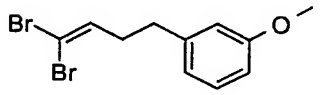
7.38 mmol) in DCM (5 mL) was added. After another 10 min. the reaction mixture was allowed to reach room temperature and was stirred at that temperature for 14h. Then, the reaction mixture was added to pentane (200 mL) to give precipitation. The mixture was filtered and the insoluble fraction was dissolved in DCM (40mL) and again precipitated with pentane (200 mL). After filtration, this was repeated once more. The combined organic phases were evaporated. This gave 2.562 g crude product as an oil that contained white crystals. NMR showed a mixture of the wanted material and $O=PPh_3$. The oil, but not the crystals, could be dissolved in 5 mL ethyl acetate. The crystals were filtered off and the mother liquor was concentrated. 2.106 g (yield: 87 %) remained and this material was free from $O=PPh_3$ according to NMR.

^{1}H NMR (300 MHz): 7.25-7.15 (m, 3H), 7.07-7.02 (d t, J_1 = 6.6 Hz, J_2 = 1.7 Hz, 1H), 2.69 (t, J =7.4 Hz, 2H), 2.38 (q, J = 7.4 Hz, 2H).

^{13}C NMR (75 MHz):142.2, 136.8, 134.0, 129.6, 128.3, 126.3, 126.3, 89.9, 34.3, 33.4.

Example 8

Preparation of 1-methoxy-3-(4,4-dibromobut-3-en-1-yl)benzene (compound 8): prepared according to method D above, with 3-(3-methoxyphenyl)propanal as starting material.



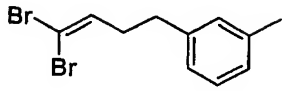
(8)

^{1}H NMR (300 MHz): 7.10 (t, J = 7.6 Hz, 2H), 6.65 (m, 2H), 6.30 (t, J = 7.2 Hz, 1H), 3.68 (s, 3H), 2.59 (t, J = 7.6 Hz, 2H), 2.30 (q, J = 7.5 Hz, 2H).

^{13}C NMR (75 MHz): 159.4, 141.8, 137.3, 129.2, 120.4, 113.9, 111.3, 89.3, 55.0, 34.4, 33.7.

Example 9

Preparation of 1-methyl-3-(4,4-dibromobut-3-en-1-yl)benzene (compound 9): prepared according to method D above, with 3-(3-methylphenyl)propanal as starting material.


(9)

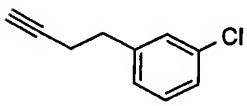
^{1}H NMR (500 MHz): 7.22 (t, J = 7.75 Hz, 1H), 7.08-7.0 (m, 3H), 6.33 (t, J = 7.2 Hz, 1H), 2.60 (t, J = 7.6 Hz, 2H), 2.32 (q, J =7.6 Hz, 2H), 2.37 (s, 3H).

^{13}C NMR (125 MHz): 140.3, 137.6, 132.6, 129.0, 128.5,126.9, 125.2, 89.3, 34.5, 33.6, 21.3.

Method E

Example 10

Preparation of 1-but-3-yn-1-yl-3-chlorobenzene (compound 10):


(10)

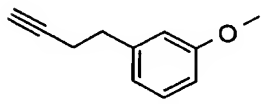
1-chloro-3-(4,4-dibromobut-3-en-1-yl)benzene (1.106 g, 3.41 mmol) was dissolved in THF (5 mL) and then cooled to –78 °C. Lithium bis(trimethylsilyl)amide (5.11 mL of a 1.0 M solution in THF, 1.5 eq.) was added dropwise and the solution was stirred for 0.5h. *n*-Butyl lithium (5.05 mL of a 1.6 M solution in hexanes, 2.5 eq.) was then added dropwise. The reaction mixture was stirred for 1h at –78 °C and then for 1h at room temperature before quenching with water (20 mL). The organic phase was separated and the water phase was extracted with Et_2O (2 x 20 mL). The combined organic phases were dried with sodium sulphate and evaporated. This gave 0.541 g (yield: 96 %) crude product.

^{1}H NMR (300 MHz): 7.24-7.15 (m, 3H), 7.11-7.05 (m, 1H), 2.79 (t, J = 7.4 Hz, 2H), 2.45 (d t, J_1 = 7.4 Hz, J_2 = 2.6 Hz, 2H), 1.97 (t, J = 2.6 Hz, 1H).

^{13}C NMR (125 MHz): 142.2, 134.0, 129.5, 128.5, 126.6, 126.4, 83.0, 69.2, 34.2, 20.2.

Example 11

Preparation of 1-but-3-yn-1-yl-3-methoxybenzene (compound 11): prepared according to method E above, with 1-methoxy-3-(4,4-dibromobut-3-en-1-yl)benzene as starting material.



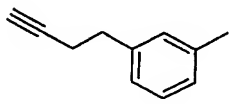
(11)

^{1}H NMR (500 MHz): 7.23 (t, J = 7.7 Hz, 1H), 6.83 (d, J = 7.7 Hz, 1H), 6.79 (m, 2H), 3.82 (s, 3H), 2.85 (t, J =7.6 Hz, 2H), 2.50 (t d, J_1 = 7.6 Hz, J_2 = 2.6 Hz, 2H), 2.00 (t, J = 2.6 Hz, 1H).

^{13}C NMR (125 MHz): 159.5, 141.9, 129.3, 120.7, 114.1, 111.5, 83.7, 68.8, 55.0, 34.8, 29.6.

Example 12

Preparation of 1-but-3-yn-1-yl-3-methylbenzene (compound 12): prepared according to method E above, with 1-methyl-3-(4,4-dibromobut-3-en-1-yl)benzene as starting material.



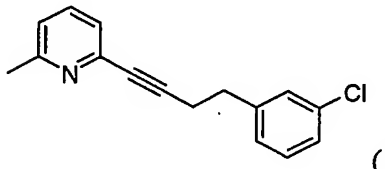
(12)

^{13}C NMR (75 MHz): 140.2, 137.8, 129.0, 128.1, 126.9, 125.2, 83.8, 68.7, 34.8, 21.4, 20.6.

Method F

Example 13

Preparation of 2-[4-(3-chlorophenyl)but-1-yn-1-yl]-6-methylpyridine (compound 13):



(13)

To 2-bromo-6-methylpyridine (0.054 g, 0.31 mmol) was added crude 1-but-3-yn-1-yl-3-chlorobenzene (0.057 g, 0.35 mmol, 1.10 eq.), followed by $(PPh_3)_2PdCl_2$ (0.007 g, 0.01 mmol, 0.03 eq.) and triethylamine (0.50 mL). The mixture was stirred under nitrogen at 0 °C for 0.5h. Then, CuI (0.002 g, 0.01 mmol, 0.03 eq.) was added and the mixture was allowed to reach room temperature over ca. 1/2h and then heated at 60 °C for 12h. The material was filtered through a 1g SiO_2 plug, while rinsing with ethyl acetate (15 mL). Flash chromatography on silica gel by elution with pentane/Et_2O 3:1, then 2:1, gave 0.027 g (Yield: 34 %, compared to 2-bromo-6-methylpyridine).

TLC: R_f (pentane/Et_2O 2:1) = 0.29.

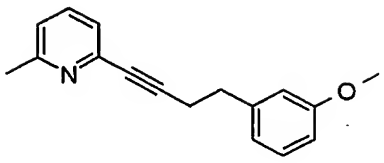
^1H NMR (300 MHz): 7.41 (t, J = 7.8 Hz, 1H), 7.21-7.03 (m, 5H), 6.97 (d, J = 7.8 Hz, 1H), 2.84 (t, J = 7.8 Hz, 2H), 2.63 (t, J = 7.8 Hz, 2H), 2.46 (s, 3H).

^{13}C NMR (75 MHz): 158.3, 142.2, 142.1, 136.2, 133.8, 129.4, 128.4, 126.4, 126.3, 123.8, 122.2, 89.2, 81.1, 34.1, 24.4, 21.3.

Example 14

Preparation of 2-[4-(3-methoxyphenyl)but-1-yn-1-yl]-6-methylpyridine (compound 14):

prepared according to method F above, with 2-bromo-6-methylpyridine and 1-but-3-yn-1-yl-3-methoxybenzene as starting materials.



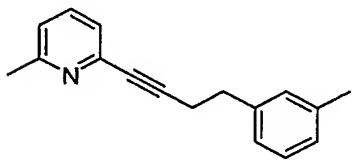
(14)

^{1}H NMR (500 MHz): 7.50 (t, J = 7.6 Hz, 1H), 2.23 (t, J = 7.8 Hz, 1H), 7.18 (d, J = 7.7 Hz, 1H), 7.06 (d, J = 7.8 Hz, 1H), 6.78 (dd, J_1 = 8.1 Hz, J_2 = 2.3 Hz, 1H), 6.87-6.81 (m, 2H), 3.80 (s, 3H), 2.94 (t, J = 7.7 Hz, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.55 (s, 3H).

^{13}C NMR (75 MHz): 159.4, 158.4, 142.7, 141.9, 136.1, 129.2, 123.7, 122.0, 120.6, 114.0, 111.6, 89.6, 81.0, 55.1, 34.8, 24.5, 21.6.

Example 15

Preparation of 2-methyl-6-[4-(3-methylphenyl)but-1-yn-1-yl]pyridine (compound 15): prepared according to method F above, with 2-bromo-6-methylpyridine and 1-but-3-yn-1-yl-3-methylbenzene as starting materials.



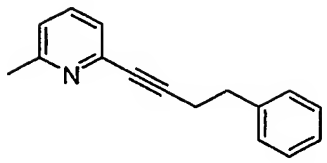
(15)

^{1}H NMR (300 MHz): 7.51 (t, J = 7.7 Hz, 1H), 7.21 (t, J = 7.4 Hz, 1H), 7.19 (t, J = 7.4 Hz, 1H), 7.10-7.01 (m, 4 H), 2.93 (t, J= 7.7 Hz, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.55 (s, 3H), 2.35 (s, 3H).

^{13}C NMR (75 MHz): 158.5, 142.8,140.3, 137.8, 136.1, 129.1 128.2, 126.9, 125.3, 123.7, 122.0, 89.6, 80.9, 34.8, 24.6, 21.7, 21.4.

Example 16

Preparation of 2-methyl-6-(4-phenylbut-1-yn-1-yl)pyridine (compound 16): prepared according to method F above with 2-bromo-6-methylpyridine and 4-phenyl-but-1-yn as starting materials.

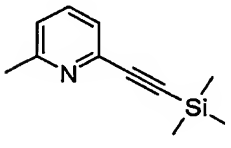


(16)

^{1}H NMR (300 MHz): 7.46 (t, J = 7.6 Hz, 1H), 7.33-7.19 (m, 5H), 7.14 (d, J = 7.7 Hz, 1H), 7.02 (d, J = 7.7 Hz, 1H), 2.95 (t, J = 7.6 Hz, 2H), 2.71 (t, J = 7.6 Hz, 2H), 2.52 (s, 3H).
^{13}C NMR (75 MHz): 158.2, 142.6, 140.1, 135.9, 128.1 (high intensity), 126.0, 123.5, 121.8, 89.3, 80.8, 34.6, 24.4, 21.5.

Example 17

2-methyl-6-[(trimethylsilyl)ethynyl]pyridine (compound 17):



(17)

6-bromo-2-methylpyridine (0.516 g, 3.0 mmol) was mixed with ethynyl(trimethyl)silane (0.324 g, 3.3 mmol, 1.10 eq.) and $(PPh_3)_2PdCl_2$ (0.063 g, 0.09 mmol, 0.03 eq.) and triethylamine (1.21g, 1.67 mL, 12.0 mmol, 4.0 eq. was added at 0 °C. The mixture was stirred for 1/2h at 0 °C before CuI (0.017 g, 0.09 mmol, 0.03 eq.) was added and the mixture was heated to room temperature over 15 min. After stirring 15 min. at room temperature it was heated to 60 °C. Heating was maintained for 2h and finally the mixture was left at room temperature for 16h. LC/MS showed that none of the bromide remained. Phosphate buffer (5 mL, 0.2 M, pH 7) was added. Extraction with DCM (3 x 5 mL) was performed by use of phase separator. The organic phases were combined and dried with sodium sulphate. After evaporation, 0.623g product was obtained. After flash chromatography on Si-gel by eluting with 5 %, later 10 % EtOAc in heptane, 0.320 g material was isolated. (Yield: 56 %).

TLC: R_f (heptane/EtOAc 2:1) = 0.56.

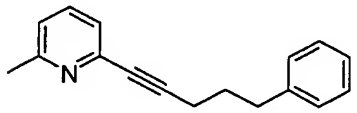
^{1}H NMR (300 MHz): 7.37 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 6.93 (d, J = 7.8 Hz, 1H), 2.40 (s, 3H), 0.14 (s, 9H).

^{13}C NMR (75 MHz): 158.2, 141.8, 135.7, 124.0, 122.3, 103.5, 93.6, 24.2, -0.51.

Example 18

2-methyl-6-(5-phenylpent-1-yn-1-yl)pyridine (compound 18):

(Methodology by A.S. Pilcher, P. DeShong, *J. Org. Chem.*, **1996**, *61*, 6901-6905)



(18)

2-methyl-6-[(trimethylsilyl)ethynyl]pyridine (0.038 g, 0.2 mmol, 2.0 eq.) was mixed with (3-bromopropyl)benzene (0.020 g, 0.1 mmol) and tetrabutylammonium triphenyldifluorosilicate (0.081 g, 0.3 mmol, 1.5 eq.) and heated at 60 °C for 24h in a sealed vial. LC/MS after that time showed the molecular weight of the product. Heating at 60 °C was continued for 24h further, but without any change in LC/MS. Purification by flash chromatography on Si with pentane/ether fractions as eluent, first 6:1, then 4:1, gave 0.008 g product. (Yield: 17 %).

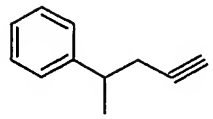
TLC: R_f (pentane/Et_2O 4:1) = 0.34.

^{1}H NMR (500 MHz): 7.50 (t, J = 7.8 Hz, 1H), 7.32-7.25 (m, 2H), 7.24-7.18 (m, 4H), 7.06 (d, J = 7.8 Hz, 1H), 2.78 (t, J = 7.8 Hz, 2H), 2.54 (s, 3H), 2.45 (t, J = 7.4 Hz, 2H), 1.96 (q, J = 7.4 Hz, 2H).

Example 19

Preparation of (1-Methyl-but-3-ynyl)-benzene

(1-Methyl-but-3-ynyl)-benzene was prepared according to method E above using (4,4-Dibromo-1-methyl-but-3-enyl)-benzene as starting material.

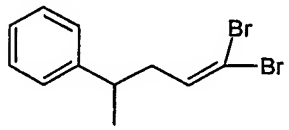


^{1}H NMR (400 MHz): 7.33 (m, 2 H), 7.25 (m, 3 H), 3.01 (m, 1 H), 2.45 (m, 2 H), 1.99 (t, 1 H), 1.41 (d, 3 H).

Example 20

Preparation of (4,4-Dibromo-1-methyl-but-3-enyl)-benzene

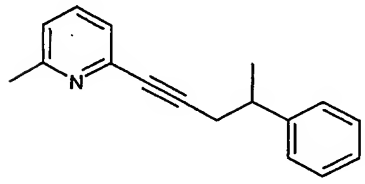
(4,4-Dibromo-1-methyl-but-3-enyl)-benzene was prepared according to method D above with 3-phenyl-butyraldehyde as starting material.



Example 21

2-methyl-6-(4-phenylpent-1-yn-1-yl)pyridine (compound 19)

The compound was prepared according to method F above using (1-Methyl-but-3-ynyl)-benzene and 2-bromo-6-methylpyridine as starting materials.



^{1}H NMR: 7.49 (t, 1H), 7.26 (m, 5H), 7.14 (d, 1H), 7.04 (d, 1H), 3.11 (m, 1H), 2.73 (dd, 1H), 2.64 (dd, 1H), 2.53 (s, 1H), 1.44 (d, 3H).

^{13}C NMR: 158.9, 145.9, 143.3, 136.4, 128.6, 127.1, 126.6, 124.1, 122.3, 89.3, 81.9, 39.2, 28.9, 24.7, 21.1.

Biological evaluation

Functional assessment of mGluR5 antagonism in cell lines expressing mGluR5d

The properties of the compounds of the invention can be analyzed using standard assays for pharmacological activity. Examples of glutamate receptor assays are well known in the art as described in for example Aramori *et al., Neuron* 8:757 (1992), Tanabe *et* al., *Neuron* 8:169 (1992), Miller *et al., J. Neuroscience* 15: 6103 (1995), Balazs, *et al., J. Neurochemistry* 69:151 (1997). The methodology described in these publications is incorporated herein by reference. Conveniently, the compounds of the invention can be studied by means of an assay (FLIPR) that measures the mobilization of intracellular calcium, $[Ca^{2+}]_i$ in cells expressing mGluR5 or another assay (IP3) that measures inositol phosphate turnover.

FLIPR Assay

Cells expressing human mGluR5d as described in WO97/05252 are seeded at a density of 100,000 cells per well on collagen coated clear bottom 96-well plates with black sides and experiments are done 24 h following seeding. All assays are done in a buffer containing 127 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 0.7 mM NaH_2PO_4, 2 mM $CaCl_2$, 0.422 mg/ml $NaHCO_3$, 2.4 mg/ml HEPES, 1.8 mg/ml glucose and 1 mg/ml BSA Fraction IV (pH 7.4). Cell cultures in the 96-well plates are loaded for 60 minutes in the above mentioned buffer containing 4 μM of the acetoxymethyl ester form of the fluorescent calcium indicator fluo-3 (Molecular Probes, Eugene, Oregon) in 0.01% pluronic acid (a proprietary, non-ionic surfactant polyol – CAS Number 9003-11-6). Following the loading period the fluo-3 buffer is removed and replaced with fresh assay buffer. FLIPR experiments are done using a laser setting of 0.800 W and a 0.4 second CCD camera shutter speed with excitation and emission wavelengths of 488 nm and 562 nm, respectively. Each experiment is initiated with 160 μl of buffer present in each well of the cell plate. A 40 μl addition from the antagonist plate was followed by a 50 μL addition from the agonist plate. A 90 second interval separates the antagonist and agonist additions. The fluorescence signal is sampled 50 times at 1 second intervals followed by 3 samples at 5 second intervals immediately after each of the two additions. Responses are measured as the difference between the peak

height of the response to agonist, less the background fluorescence within the sample period. IC_{50} determinations are made using a linear least squares fitting program.

IP3 Assay

An additional functional assay for mGluR5d is described in WO97/05252 and is based on phosphatidylinositol turnover. Receptor activation stimulates phospholipase C activity and leads to increased formation of inositol 1,4,5,triphosphate (IP_3).

GHEK stably expressing the human mGluR5d are seeded onto 24 well poly-L-lysine coated plates at 40 x 10^4 cells /well in media containing 1 μCi/well [3H] myo-inositol. Cells were incubated overnight (16 h), then washed three times and incubated for 1 h at 37°C in HEPES buffered saline (146 mM NaCl, 4.2 mM KCl, 0.5 mM $MgCl_2$, 0.1% glucose, 20 mM HEPES, pH 7.4) supplemented with 1 unit/ml glutamate pyruvate transaminase and 2 mM pyruvate. Cells are washed once in HEPES buffered saline and pre-incubated for 10 min in HEPES buffered saline containing 10 mM LiCl. Compounds are incubated in duplicate at 37°C for 15 min, then either glutamate (80 μM) or DHPG (30 μM) is added and incubated for an additional 30 min. The reaction is terminated by the addition of 0.5 ml perchloric acid (5%) on ice, with incubation at 4°C for at least 30 min. Samples are collected in 15 ml polypropylene tubes and inositol phosphates are separated using ion-exchange resin (Dowex AG1-X8 formate form, 200-400 mesh, BIORAD) columns. Inositol phosphate separation was done by first eluting glycero phosphatidyl inositol with 8 ml 30 mM ammonium formate. Next, total inositol phosphates is eluted with 8 ml 700 mM ammonium formate / 100 mM formic acid and collected in scintillation vials. This eluate is then mixed with 8 ml of scintillant and [3H] inositol incorporation is determined by scintillation counting. The dpm counts from the duplicate samples are plotted and IC_{50} determinations are generated using a linear least squares fitting program.

Abbreviations

BSA	Bovine Serum Albumin
CCD	Charge Coupled Device

CRC	Concentration Response Curve
DHPG	3,5-dihydroxyphenylglycine
DPM	Disintegrations per Minute
EDTA	Ethylene Diamine Tetraacetic Acid
FLIPR	Fluorometric Imaging Plate reader
GHEK	GLAST-containing Human Embrionic Kidney
GLAST	glutamate/aspartate transporter
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (buffer)
IP_3	inositol triphosphate

Generally, the compounds are active in the assay above with IC_{50} values less than 10 000 nM. In one aspect of the invention, the IC_{50} value is less than 1 μM. In a further aspect of the invention, the IC_{50} value is less than 100 nM.

Screening for compounds active against TLESR

Adult Labrador retrievers of both genders, trained to stand in a Pavlov sling, are used. Mucosa-to-skin esophagostomies are formed and the dogs are allowed to recover completely before any experiments are done.

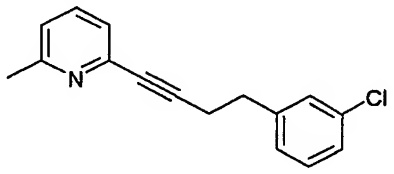
Motility measurement

In brief, after fasting for approximately 17 h with free supply of water, a multilumen sleeve/sidehole assembly (Dentsleeve, Adelaide, South Australia) is introduced through the esophagostomy to measure gastric, lower esophageal sphincter (LES) and esophageal pressures. The assembly is perfused with water using a low-compliance manometric perfusion pump (Dentsleeve, Adelaide, South Australia). An air-perfused tube is passed in the oral direction to measure swallows, and an antimony electrode monitored pH, 3 cm above the LES. All signals are amplified and acquired on a personal computer at 10 Hz.

When a baseline measurement free from fasting gastric/LES phase III motor activity has been obtained, placebo (0.9% NaCl) or test compound is administered intravenously (i.v., 0.5 ml/kg) in a foreleg vein. Ten min after i.v. administration, a nutrient meal (10% peptone, 5% D-glucose, 5% Intralipid, pH 3.0) is infused into the stomach through the central lumen of the assembly at 100 ml/min to a final volume of 30 ml/kg. The infusion of the nutrient meal is followed by air infusion at a rate of 500 ml/min until an intragastric pressure of 10±1 mmHg is obtained. The pressure is then maintained at this level throughout the experiment using the infusion pump for further air infusion or for venting air from the stomach. The experimental time from start of nutrient infusion to end of air insufflation is 45 min. The procedure has been validated as a reliable means of triggering TLESRs.

TLESRs is defined as a decrease in lower esophageal sphincter pressure (with reference to intragastric pressure) at a rate of >1 mmHg/s. The relaxation should not be preceded by a pharyngeal signal ≤2s before its onset in which case the relaxation is classified as swallow-induced. The pressure difference between the LES and the stomach should be less than 2 mmHg, and the duration of the complete relaxation longer than 1 s.

Biological Evaluation Example 1



2-[4-(3-chlorophenyl)but-1-yn-1-yl]-6-methylpyridine (compound 13) was prepared according to the procedure in example 13 above. 2-[4-(3-chlorophenyl)but-1-yn-1-yl]-6-methylpyridine was tested on adult Labrador retrievers of both genders in accordance with the barostat model described above.

Table 1.1 – barostat model

Compound	DOSE [μmol/kg/h] *infusion during 60 min*	% INHIBITION ± SEM (N)
Compound 13	4	31 ± 13 (4)

N= number of dogs tested.